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         (c) 2006 The Gale Group
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         (c) format only 2006 Dialog
  File 444:New England Journal of Med. 1985-2006/Sep W3
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         (c)2006 The Thomson Corp.
  File  94:JICST-EPlus 1985-2006/Jun W3
         (c)2006 Japan Science and Tech Corp(JST)

      Set  Items  Description
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Terminal set to DLINK
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Set     Items   Description
S1        954   RECOMBINANT? (10N) (BOTULIN? OR BOTOX OR BONT OR BOTULIS?)
S2        908   S1/1995:2006
S3         46   S1 NOT S2
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3/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10295821 PMID: 7530992
Effect of botulinum C3 exoenzyme on cell growth and cytoskeleton organization in transformed human epidermal cells in culture: a possible role for rho protein in epidermal cells.
Yamamoto M; Morii N; Ikai K; Imamura S
Department of Dermatology, Kyoto University Faculty of Medicine, Japan.
Journal of dermatological science (IRELAND) Oct 1994, 8 (2) p103-9, ISSN 0923-1811--Print Journal Code: 9011485
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
We examined the role of rho gene products (rho proteins) on cell growth and cytoskeleton organization in transformed human epidermal cells in culture (HSC-1), using **recombinant botulinum** C3 exoenzyme which specifically ADP-ribosylates rho proteins. Incubation of HSC-1 cell lysates with C3 exoenzyme revealed a single [32P]ADP-ribosylated protein with a molecular weight of 23,000. This protein was identified as rhoA protein by isoelectric focusing (pI 6.0). Addition of C3 exoenzyme to the culture medium of HSC-1 cells changed the shape of HSC-1 cells to a round form with beaded processes in a time- and dose-dependent manner. Moreover, C3 treatment reduced the cell growth rate; 72-h treatment with C3 exoenzyme at 1, 3, 10, 30 and 60 micrograms/ml culture medium resulted in 9.0 +/- 1.8%, 20 +/- 2.9%, 26 +/- 2.3%, 50 +/- 1.4% and 40 +/- 2.0% inhibition of the growth rate relative to controls, respectively. Under this condition, actin stress fibers were disassembled, as revealed using fluorescent-labeled phallacidin, whereas keratin intermediate filaments were not affected, visualized by immunofluorescence using anti-keratin antibody. These results suggest that rho proteins are closely related to cell growth and that these proteins regulate, at least in part, the assembly of actin stress fibers in transformed human epidermal cells.
Descriptors: *ADP Ribose Transferases--pharmacology--PD; *Botulinum Toxins; *Cytoskeleton--drug effects--DE; *Epidermis--cytology--CY; *Epidermis--physiology--PH; *GTP-Binding Proteins--physiology--PH; Actins --ultrastructure--UL; Adenosine Diphosphate Ribose--metabolism--ME; Cell Division--drug effects--DE; Cell Line, Transformed; Epidermis --ultrastructure--UL; Humans; Keratin--ultrastructure--UL; Research Support, Non-U.S. Gov't; Tumor Cells, Cultured; rhoA GTP-Binding Protein
CAS Registry No.: 0 (Actins); 0 (Botulinum Toxins); 20762-30-5 (Adenosine Diphosphate Ribose); 68238-35-7 (Keratin)
Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP-Binding Proteins) ; EC 3.6.5.2 (rhoA GTP-Binding Protein)
Record Date Created: 19950303
Record Date Completed: 19950303

3/9/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10218916 PMID: 7962205
Probing the action of Clostridium difficile toxin B in Xenopus laevis

oocytes.
Just I; Richter H P; Prepens U; von Eichel-Streiber C; Aktories K
Institut fur Pharmakologie und Toxikologie, Universitat des Saarlandes, Homburg/Saar, Germany.
Journal of cell science (ENGLAND) Jun 1994, 107 (Pt 6) p1653-9, ISSN 0021-9533--Print Journal Code: 0052457
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Clostridium difficile toxin B and Clostridium botulinum C3 exoenzyme caused comparable morphological alteration of CHO cells, which was accompanied by disaggregation of the microfilamental cytoskeleton. The cytotoxic effect of toxin B was correlated with a decrease in C3-catalyzed ADP-ribosylation of the low-molecular-mass GTP-binding protein Rho, which is involved in the regulation of the actin cytoskeleton. We used Xenopus laevis oocytes as a model to study the toxin effect on Rho in more detail. Toxin B treatment of oocytes caused a decrease in subsequent ADP-ribosylation of cytoplasmic Rho by C3. This decrease was observed when toxin B was applied externally or after microinjection. Besides endogenous Rho, microinjected recombinant Rho-glutathione S-transferase fusion protein was affected. Impaired ADP-ribosylation of Rho was neither due to altered guanine nucleotide binding nor to complexation with the guanine nucleotide dissociation inhibitor, which is known to inactivate Rho and to prevent Rho modification by C3. Proteolytical degradation of Rho was excluded by immunoblot analysis. In intact oocytes toxin B caused neither ADP-ribosylation nor phosphorylation of Rho. The data indicate that C. difficile toxin B acts on Rho proteins in Xenopus oocytes to inhibit ADP-ribosylation by C3. It is suggested that toxin B mediates its cytotoxic effect via functional inactivation of Rho.
Descriptors: *Bacterial Proteins; *Bacterial Toxins--pharmacology--PD; *Botulinum Toxins; *GTP-Binding Proteins--metabolism--ME; *Oocytes--drug effects--DE; ADP Ribose Transferases--antagonists and inhibitors--AI; ADP Ribose Transferases--pharmacology--PD; Animals; CHO Cells--drug effects--DE ; Comparative Study; Cricetinae; Cytoskeleton--drug effects--DE; Microfilaments--drug effects--DE; Microinjections; Poly(ADP-ribose) Polymerases--antagonists and inhibitors--AI; Recombinant Fusion Proteins --pharmacology--PD; Research Support, Non-U.S. Gov't; Xenopus laevis
CAS Registry No.: 0 (Bacterial Proteins); 0 (Bacterial Toxins); 0 (Botulinum Toxins); 0 (Recombinant Fusion Proteins); 0 (toxB protein, Clostridium difficile)
Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 2.4.2.30 (Poly(ADP-ribose) Polymerases); EC 3.6.1.- (GTP-Binding Proteins)
Record Date Created: 19941129
Record Date Completed: 19941129

3/9/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10210854 PMID: 7954816
The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells.
Chong L D; Traynor-Kaplan A; Bokoch G M; Schwartz M A
Department of Immunology, Scripps Research Institute, La Jolla, California 92037.
Cell (UNITED STATES) Nov 4 1994, 79 (3) p507-13, ISSN 0092-8674--

Print Journal Code: 0413066
Contract/Grant No.: PO1 HL48728; HL; NHLBI; RO1 GM4428; GM; NIGMS; RO1 GM47214; GM; NIGMS

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Integrin-mediated adhesion is known to stimulate production of phosphatidylinositol 4,5-bisphosphate (4,5-PIP2) and increase 4,5-PIP2 hydrolysis in response to platelet-derived growth factor (PDGF). We now show that treatment of cells with lovastatin, which inhibits modification of small GTP-binding proteins, reduced PIP2 levels and decreased calcium mobilization in response to PDGF and thrombin. In cell lysates, GTP gamma S stimulated PIP 5-kinase activity, and this effect was blocked by **botulinum** C3 exoenzyme, suggesting that Rho was responsible. GTP-bound **recombinant** Rho stimulated PIP 5-kinase activity, whereas GDP-Rho was much less potent and GTP-bound Rac was ineffective. Microinjected botulinum C3 exoenzyme caused diminished calcium mobilization in response to PDGF or thrombin. Conversely, microinjection of activated Rho reversed the decrease in calcium mobilization normally seen in nonadherent cells. These data demonstrate that Rho regulates 4,5-PIP2 synthesis and, indirectly, 4,5-PIP2 hydrolysis. They also raise the possibility that PIP2 synthesis could mediate the effects of Rho on the actin cytoskeleton.

Descriptors: *Botulinum Toxins; *Drosophila Proteins; *GTP-Binding Proteins--metabolism--ME; *Membrane Proteins--metabolism--ME; *Phosphotransferases (Alcohol Group Acceptor)--metabolism--ME; *Signal Transduction; ADP Ribose Transferases--pharmacology--PD; Animals; Calcium--metabolism--ME; Cell Adhesion--physiology--PH; Cells, Cultured; Fibroblasts; GTP-Binding Proteins--genetics--GE; Guanosine 5'-O-(3-Thiotriphosphate)--pharmacology--PD; Integrins--physiology--PH; Lovastatin--pharmacology--PD; Mice; Microinjections; Phosphatidylinositol 4,5-Diphosphate; Phosphatidylinositol Phosphates--metabolism--ME; Platelet-Derived Growth Factor--pharmacology--PD; Recombinant Proteins--metabolism--ME; Research Support, U.S. Gov't, P.H.S.; Thrombin--pharmacology--PD

CAS Registry No.: 0 (Botulinum Toxins); 0 (Drosophila Proteins); 0 (Integrins); 0 (Membrane Proteins); 0 (Phosphatidylinositol 4,5-Diphosphate); 0 (Phosphatidylinositol Phosphates); 0 (Platelet-Derived Growth Factor); 0 (Recombinant Proteins); 0 (Rho protein, Drosophila); 0 (rho-2 protein, Drosophila); 37589-80-3 (Guanosine 5'-O-(3-Thiotriphosphate)); 7440-70-2 (Calcium); 75330-75-5 (Lovastatin)

Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 2.7.1 (Phosphotransferases (Alcohol Group Acceptor)); EC 2.7.1.68 (1-phosphatidylinositol-4-phosphate 5-kinase); EC 3.4.21.5 (Thrombin); EC 3.6.1.- (GTP-Binding Proteins)

Record Date Created: 19941220
Record Date Completed: 19941220

3/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10061598 PMID: 8198524

ADP-ribosylation of Rho proteins by Clostridium botulinum exoenzyme C3 is influenced by phosphorylation of Rho-associated factors.

Fritz G; Aktories K
Institut fur Pharmakologie und Toxikologie, Universitat des Saarlandes,

Homburg, Germany.
Biochemical journal (ENGLAND) May 15 1994, 300 (Pt 1) p133-9, ISSN 0264-6021--Print Journal Code: 2984726R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Specific [32P]ADP-ribosylation by Clostridium botulinum exoenzyme C3 was used to study the involvement of phosphorylation in the regulation of the low-molecular-mass GTP-binding protein Rho. Dephosphorylation of CHO cell extracts by alkaline phosphatase treatment resulted in a 80-90% reduction in the C3-catalysed [32P]ADP-ribosylation of Rho proteins in both cytosolic and membrane fractions. Similar results were obtained after dephosphorylation with protein phosphatase type-1 from bovine retina, whereas type-2B and type-2C phosphatases had no effect on the level of subsequent [32P]ADP-ribosylation of Rho by C3. Incubation of CHO cell lysate under phosphorylation conditions increased the subsequent C3-mediated [32P]ADP-ribosylation of Rho proteins. The protein kinase inhibitors H7 and H9 had no effect on [32P]ADP-ribosylation at concentrations which are specific for inhibition of protein kinase A or C. Recombinant glutathione S-transferase-RhoA fusion protein (GST-RhoA) was phosphorylated by protein kinase A; however, the phosphorylation had no stimulatory effect on the ADP-ribosylation of GST-RhoA by C3. An approx. 48 kDa phosphoprotein was identified which bound specifically to recombinant GST-RhoA fusion protein. By gel-permeation chromatography, Rho-containing complexes of approx. 50 kDa and 130-170 kDa were detected. The ADP-ribosylation of Rho in the 130-170 kDa complex was reduced by alkaline phosphatase pretreatment. The data suggest that Rho activity is influenced by phosphorylation of Rho-associated regulatory factors. Phosphorylation/dephosphorylation of these Rho-regulating factors appears to alter the ability of Rho to serve as a substrate for C3-induced [32P]ADP-ribosylation.

Descriptors: *ADP Ribose Transferases--metabolism--ME; *Adenosine Diphosphate Ribose--metabolism--ME; *Botulinum Toxins; *GTP-Binding Proteins--metabolism--ME; *Guanine Nucleotide Dissociation Inhibitors; 3T3 Cells; Animals; Binding Sites; Biological Factors--metabolism--ME; CHO Cells; Cricetinae; Mice; Phosphoproteins--metabolism--ME; Phosphorylation; Recombinant Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; rhoA GTP-Binding Protein

CAS Registry No.: 0 (Biological Factors); 0 (Botulinum Toxins); 0 (Guanine Nucleotide Dissociation Inhibitors); 0 (Phosphoproteins); 0 (Recombinant Proteins); 133312-85-3 (rhoB p20 GDI); 20762-30-5 (Adenosine Diphosphate Ribose)

Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rhoA GTP-Binding Protein)

Record Date Created: 19940628
Record Date Completed: 19940628

3/9/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09817440 PMID: 8243676

Botulinum neurotoxins serotypes A and E cleave SNAP-25 at distinct COOH-terminal peptide bonds.

Schiavo G; Santucci A; Dasgupta B R; Mehta P P; Jontes J; Benfenati F; Wilson M C; Montecucco C

Centro CNR Biomembrane, Universita di Padova, Italy.
FEBS letters (NETHERLANDS) Nov 29 1993, 335 (1) p99-103, ISSN 0014-5793--Print Journal Code: 0155157
Contract/Grant No.: NS17742; NS; NINDS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

SNAP-25, a membrane-associated protein of the nerve terminal, is specifically cleaved by botulinum neurotoxins serotypes A and E, which cause human and animal botulism by blocking neurotransmitter release at the neuromuscular junction. Here we show that these two metallo-endopeptidase toxins cleave SNAP-25 at two distinct carboxyl-terminal sites. Serotype A catalyses the hydrolysis of the Gln197-Arg198 peptide bond, while serotype E cleaves the Arg180-Ile181 peptide lineage. These results indicate that the carboxyl-terminal region of SNAP-25 plays a crucial role in the multi-protein complex that mediates vesicle docking and fusion at the nerve terminal.

Descriptors: *Botulinum Toxins--metabolism--ME; *Membrane Proteins; *Nerve Tissue Proteins--metabolism--ME; Amino Acid Sequence; Animals; Binding Sites; Brain Chemistry; Hydrolysis; Immunoblotting; Molecular Sequence Data; Nerve Tissue Proteins--chemistry--CH; Peptide Fragments --chemistry--CH; Peptide Fragments--isolation and purification--IP; Peptide Fragments--metabolism--ME; Rats; Recombinant Proteins--metabolism --ME; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.; Synaptosomal-Associated Protein 25; Synaptosomes--chemistry--CH

CAS Registry No.: 0 (Botulinum Toxins); 0 (Membrane Proteins); 0 (Nerve Tissue Proteins); 0 (Peptide Fragments); 0 (Recombinant Proteins); 0 (Snap25 protein, rat); 0 (Synaptosomal-Associated Protein 25)

Record Date Created: 19931229
Record Date Completed: 19931229

3/9/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09745618 PMID: 8103915
Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25.
Blasi J; Chapman E R; Link E; Binz T; Yamasaki S; De Camilli P; Sudhof T C; Niemann H; Jahn R
Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510.
Nature (ENGLAND) Sep 9 1993, 365 (6442) p160-3, ISSN 0028-0836--Print Journal Code: 0410462
Publishing Model Print; Comment in Nature. 1993 Sep 9;365(6442) 104-5; Comment in PMID 8103914
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Neurotransmitter release is potently blocked by a group of structurally related toxin proteins produced by Clostridium botulinum. Botulinum neurotoxin type B (BoNT/B) and tetanus toxin (TeTx) are zinc-dependent proteases that specifically cleave synaptobrevin (VAMP), a membrane protein of synaptic vesicles. Here we report that inhibition of transmitter release

from synaptosomes caused by botulinum neurotoxin A (BoNT/A) is associated with the selective proteolysis of the synaptic protein SNAP-25. Furthermore, isolated or **recombinant** L chain of **BoNT** /A cleaves SNAP-25 in vitro. Cleavage occurred near the carboxyterminus and was sensitive to divalent cation chelators. In addition, a glutamate residue in the BoNT/A L chain, presumably required to stabilize a water molecule in the zinc-containing catalytic centre, was required for proteolytic activity. These findings demonstrate that BoNT/A acts as a zinc-dependent protease that selectively cleaves SNAP-25. Thus, a second component of the putative fusion complex mediating synaptic vesicle exocytosis is targeted by a clostridial neurotoxin.

Descriptors: *Botulinum Toxins--pharmacology--PD; *Membrane Proteins; *Nerve Tissue Proteins--metabolism--ME; Glutamates--metabolism--ME; Glutamic Acid; In Vitro; Neurotransmitter Agents--metabolism--ME; Research Support, Non-U.S. Gov't; Synaptic Membranes--metabolism--ME; Synaptosomal-Associated Protein 25; Synaptosomes--metabolism--ME; Tetanus Toxin--pharmacology--PD

CAS Registry No.: 0 (Botulinum Toxins); 0 (Glutamates); 0 (Membrane Proteins); 0 (Nerve Tissue Proteins); 0 (Neurotransmitter Agents); 0 (Synaptosomal-Associated Protein 25); 0 (Tetanus Toxin); 56-86-0 (Glutamic Acid)

Record Date Created: 19931008

Record Date Completed: 19931008

3/9/7 (Item 7 from file: 155)

09724873 PMID: 8355622

Similarity in nucleotide sequence of the gene encoding nontoxic component of botulinum toxin produced by toxigenic Clostridium butyricum strain BL6340 and Clostridium botulinum type E strain Mashike.

Fujii N; Kimura K; Yokosawa N; Oguma K; Yashiki T; Takeshi K; Ohyama T; Isogai E; Isogai H

Department of Microbiology, School of Medicine, Sapporo Medical University, Hokkaido, Japan.

Microbiology and immunology (JAPAN) 1993, 37 (5) p395-8, ISSN 0385-5600--Print Journal Code: 7703966

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor toxin is determined in **recombinant** plasmid pU9BUH containing about 6.0 kb HindIII fragment obtained from chromosomal DNA of Clostridium butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The molecular weight calculated from deduced amino acid residues is estimated 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinum type E strain Mashike. This corresponds to the difference of 17 amino acid residues in these nontoxic component.

Descriptors: *Botulinum Toxins--genetics--GE; *Clostridium--genetics--GE; *Clostridium botulinum--genetics--GE; Amino Acid Sequence; Base Sequence; Cloning, Molecular; Clostridium--classification--CL; DNA, Bacterial --genetics--GE; Gene Expression; Molecular Sequence Data; Molecular Weight; Open Reading Frames; Plasmids--genetics--GE; Sequence Homology, Amino Acid;

Sequence Homology, Nucleic Acid
Molecular Sequence Databank No.: GENBANK/D12739
CAS Registry No.: 0 (Botulinum Toxins); 0 (DNA, Bacterial); 0 (Plasmids)
Record Date Created: 19930921
Record Date Completed: 19930921

3/9/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09610076 PMID: 8385945
Enhancement of Clostridium botulinum **C3-catalysed ADP-ribosylation of** recombinant **rhoA by sodium dodecyl sulfate.**
Just I; Mohr C; Habermann B; Koch G; Aktories K
Institut fur Pharmakologie und Toxikologie, Universitat des Saarlandes, Homburg, Federal Republic of Germany.
Biochemical pharmacology (ENGLAND) Apr 6 1993, 45 (7) p1409-16, ISSN 0006-2952--Print Journal Code: 0101032
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
The influence of sodium dodecyl sulfate (SDS) on ADP-ribosylation by Clostridium botulinum C3 exoenzyme (C3) was studied. SDS increased the ADP-ribosylation of recombinant rhoA and human platelet cytosolic proteins maximally at 0.01% whereas higher concentrations of the detergent (> 0.01%) inhibited the ADP-ribosylation. In contrast, ADP-ribosylation of human platelet membranes and of recombinant rhoB was inhibited by the detergent. The Km for NAD of the ADP-ribosylation of rhoA was decreased by SDS from about 10 to 0.6 microM. Whereas in the absence of SDS, the C3-induced ADP-ribosylation of recombinant rhoA is not affected by the amphiphilic wasp venom mastoparan, in the presence of SDS (0.01%) mastoparan (100 microM) inhibited the ADP-ribosylation. C3-associated NAD-glycohydrolase activity was maximally and half-maximally inhibited by 0.1 and 0.013% SDS, respectively. Inhibition of NAD-glycohydrolase activity was reversed by diluting out SDS indicating that C3 was not irreversibly denatured by SDS treatment. SDS (0.01%) completely inhibited the [3H]GTP binding of rhoA whereas the release of previously bound nucleotide was not affected. The data indicate that changes in the lipophilicity of rhoA protein largely affect its ability to serve as a substrate for C3-like ADP-ribosyltransferases.
Descriptors: *ADP Ribose Transferases--metabolism--ME; *Botulinum Toxins; *Clostridium botulinum--enzymology--EN; *GTP-Binding Proteins--genetics--GE ; *Sodium Dodecyl Sulfate--pharmacology--PD; Animals; Blood Platelets--drug effects--DE; Blood Platelets--metabolism--ME; Comparative Study; Dose-Response Relationship, Drug; GTP-Binding Proteins--metabolism--ME; Humans; Membrane Proteins--genetics--GE; NAD+ Nucleosidase--antagonists and inhibitors--AI; Peptides; Poly(ADP-ribose) Polymerases --antagonists and inhibitors--AI; Poly(ADP-ribose) Polymerases--metabolism--ME; Recombinant Proteins--genetics--GE; Research Support, Non-U.S. Gov't; Swine; Wasp Venoms--pharmacology--PD; rhoA GTP-Binding Protein; rhoB GTP-Binding Protein
CAS Registry No.: 0 (Botulinum Toxins); 0 (Membrane Proteins); 0 (Peptides); 0 (Recombinant Proteins); 0 (Wasp Venoms); 151-21-3 (Sodium Dodecyl Sulfate); 72093-21-1 (mastoparan)
Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 2.4.2.30 (Poly(ADP-ribose)

Polymerases); EC 3.2.2.5 (NAD+ Nucleosidase); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rhoA GTP-Binding Protein); EC 3.6.5.2 (rhoB GTP-Binding Protein)
Record Date Created: 19930510
Record Date Completed: 19930510

3/9/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09593572 PMID: 8458345
A chimeric toxin to study the role of the 21 kDa GTP binding protein rho in the control of actin microfilament assembly.
Aullo P; Giry M; Olsnes S; Popoff M R; Kocks C; Boquet P
Unite des Toxines Microbiennes URA CNRS 557, Institut Pasteur, Paris, France.
EMBO journal (ENGLAND) Mar 1993, 12 (3) p921-31, ISSN 0261-4189--Print Journal Code: 8208664
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
We have developed a new tool for studying the role of rho in actin stress fibre formation. Clostridium botulinum exoenzyme C3 which affects actin microfilament assembly by ADP-ribosylation of p21 rho was genetically fused in various ways to diphtheria toxin (DT). The resulting chimeric toxins were tested on Vero cells. Chimeras of C3 and both the A and B fragments of diphtheria toxin had reduced cell binding activities but were apparently able to penetrate into Vero cells by the same mechanism as DT. Upon exposure to low pH, DC3B, a fusion protein of C3 and DT B fragment, had a high affinity for the DT receptor, but was apparently not able to translocate to the cytosol upon acidification. In spite of this, addition of picomolar concentrations of DC3B to the growth medium caused disruption of the cell microfilament system associated with vinculin and blocked cell growth efficiently, indicating that the C3 part of DC3B reached the cytosol, albeit by a different mechanism than that of whole diphtheria toxin. The chimeric DC3B toxin was also applied to Vero cells infected by Listeria monocytogenes, a pathogenic bacterium that uses an unknown mechanism of actin polymerization to move rapidly in the cytosol. DC3B inhibited the bacterially induced microfilament assembly indicating that L. monocytogenes utilizes a cellular rho dependent mechanism in this process.
Descriptors: *ADP Ribose Transferases--diagnostic use--DU; *Actins --metabolism--ME; *Botulinum Toxins; *Diphtheria Toxin--diagnostic use--DU; *GTP-Binding Proteins--physiology--PH; *Recombinant Fusion Proteins --diagnostic use--DU; ADP Ribose Transferases--genetics--GE; Animals; Cercopithecus aethiops; Cloning, Molecular; Diphtheria Toxin--genetics--GE; Escherichia coli; Listeria monocytogenes--isolation and purification--IP; Mice; Recombinant Fusion Proteins--genetics--GE; Research Support, Non-U.S. Gov't; Vero Cells; Vinculin--metabolism--ME; rho GTP-Binding Proteins
CAS Registry No.: 0 (Actins); 0 (Botulinum Toxins); 0 (Diphtheria Toxin); 0 (Recombinant Fusion Proteins); 125361-02-6 (Vinculin)
Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rho GTP-Binding Proteins)
Record Date Created: 19930423
Record Date Completed: 19930423

3/9/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09294430 PMID: 1632782

Posttranslational isoprenylation of rho protein is a prerequisite for its interaction with mastoparan and other amphiphilic agents.

Koch G; Mohr C; Just I; Aktories K
Institut fur Pharmakologie und Toxikologie, Universitat des Saarlandes, Homburg, Germany.

Biochemical and biophysical research communications (UNITED STATES) Jul 15 1992, 186 (1) p448-54, ISSN 0006-291X--Print Journal Code: 0372516
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The amphiphilic agents melittin, compound 48/80 and mastoparan inhibit ADP-ribosylation of porcine brain rho protein by Clostridium **botulinum** exoenzyme C3. However, ADP-ribosylation of **recombinant** rhoA expressed in E.coli was not inhibited by these agents. Accordingly, steady state GTP hydrolysis by recombinant rhoA was not stimulated by mastoparan, whereas GTP hydrolysis by porcine brain rho was stimulated 2.5-fold in the presence of this wasp venom. After microinjection of recombinant rhoA into Xenopus laevis oocytes the inhibitory effect of mastoparan on C3 ADP-ribosylation was restored. The data suggest that the amphiphilic agents tested are only active at the posttranslationally processed form of rho and that they exert their effects via the C-terminal end.

Descriptors: *Adenosine Diphosphate Ribose--metabolism--ME; *GTP-Binding Proteins--metabolism--ME; *Hemiterpenes; *Melitten--pharmacology--PD; *NAD --metabolism--ME; *Organophosphorus Compounds--metabolism--ME; *Protein Processing, Post-Translational; *Wasp Venoms--pharmacology--PD; *p-Methoxy-N-methylphenethylamine--pharmacology--PD; Animals; Autoradiography; Brain--metabolism--ME; Carbon Radioisotopes; Cloning, Molecular; Comparative Study; Cytosol--metabolism--ME; Escherichia coli--genetics--GE; GTP-Binding Proteins--genetics--GE; GTP-Binding Proteins--isolation and purification--IP; Guanosine Triphosphate--metabolism--ME; Oocytes --metabolism--ME; Peptides; Recombinant Proteins --isolation and purification--IP; Recombinant Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; Swine; Xenopus laevis; rhoA GTP-Binding Protein
CAS Registry No.: 0 (Carbon Radioisotopes); 0 (Hemiterpenes); 0 (Organophosphorus Compounds); 0 (Peptides); 0 (Recombinant Proteins); 0 (Wasp Venoms); 20449-79-0 (Melitten); 20762-30-5 (Adenosine Diphosphate Ribose); 358-71-4 (isopentenyl pyrophosphate); 4091-50-3 (p-Methoxy-N-methylphenethylamine); 53-84-9 (NAD); 72093-21-1 (mastoparan); 86-01-1 (Guanosine Triphosphate)
Enzyme No.: EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rhoA GTP-Binding Protein)
Gene Symbol: rhoA
Record Date Created: 19920814
Record Date Completed: 19920814

3/9/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09253478 PMID: 1601841

ADP-ribosylation of the ras-related, GTP-binding protein RhoA inhibits lymphocyte-mediated cytotoxicity.

Lang P; Guizani L; Vitte-Mony I; Stancou R; Dorseuil O; Gacon G; Bertoglio J

Institut National de la Sante et de la Recherche Medicale (INSERM), Unite 333, Institut Gustave Roussy, Villejuif, France.

Journal of biological chemistry (UNITED STATES) Jun 15 1992, 267 (17) p11677-80, ISSN 0021-9258--Print Journal Code: 2985121R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

The Rho proteins are identified as a subgroup of the Ras superfamily of low molecular weight GTP-binding proteins. We have studied the expression of these proteins in human cytotoxic natural killer cells and found that RhoA is the most abundantly expressed member of the Rho family. The Rho proteins are specific substrates for ADP-ribosylation catalyzed by the C3 exoenzyme from Clostridium **botulinum** . We report here that introduction of **recombinant** C3 in electropermeabilized natural killer cells or in cytotoxic T lymphocytes resulted in a dose-dependent inhibition of their cytolytic function. Furthermore, a single substrate is efficiently ADP-ribosylated by C3 in extracts from cytotoxic cells. Biochemical analyses indicate that this substrate is RhoA, and subcellular fractionation experiments demonstrate that it is essentially present in the cytosol of the cells. Western blot analysis, however, revealed that a small proportion of the Rho protein can be found associated with the cell membrane as well as with the cytotoxic granules. These results indicate that the low molecular weight GTP-binding protein RhoA is present in cytotoxic lymphocytes and plays a critical role in cell-mediated cytotoxicity.

Descriptors: *Adenosine Diphosphate Ribose--metabolism--ME; *Botulinum Toxins; *Cytotoxicity, Immunologic; *GTP-Binding Proteins--physiology--PH; *T-Lymphocytes, Cytotoxic--immunology--IM; ADP Ribose Transferases --genetics--GE; ADP Ribose Transferases--metabolism--ME; Blotting, Northern; Blotting, Western; Cells, Cultured; Clostridium botulinum --enzymology--EN; Electrophoresis, Gel, Two-Dimensional; GTP-Binding Proteins--genetics--GE; GTP-Binding Proteins--metabolism--ME; Guanosine Triphosphate--metabolism--ME; Humans; Killer Cells, Natural--immunology--IM ; Poly(ADP-ribose) Polymerases--metabolism--ME; Research Support, Non-U.S. Gov't; Substrate Specificity; rhoA GTP-Binding Protein

CAS Registry No.: 0 (Botulinum Toxins); 20762-30-5 (Adenosine Diphosphate Ribose); 86-01-1 (Guanosine Triphosphate)

Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 2.4.2.30 (Poly(ADP-ribose) Polymerases); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rhoA GTP-Binding Protein)

Record Date Created: 19920716

Record Date Completed: 19920716

3/9/12 (Item 12 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09220648 PMID: 1577256

Cloning of a Clostridium botulinum type B toxin gene fragment encoding the N-terminus of the heavy chain.

Jung H H; Rhee S D; Yang K H

Department of Life Science, Korea Advanced Institute of Science and Technology, Taejon, Korea.

FEMS microbiology letters (NETHERLANDS) Feb 1 1992, 70 (1) p69-72,

ISSN 0378-1097--Print Journal Code: 7705721
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Two lambda gt11 clones of the toxin gene of Clostridium botulinum type B were identified by the monoclonal antibody specific to the heavy chain of type B toxin. Neither of the expressed fusion proteins from the lysates of lysogenic E. coli Y1089 showed any botulinal toxic activity. One of the clones hybridized to the oligonucleotide probe which was synthesized according to the amino acid sequence of N-terminus of heavy chain. The sequence analysis revealed that highly homologous regions in N-terminus of heavy chain exist among botulinum neurotoxins (type A, B) and tetanus toxin on the amino acid sequence level.

Descriptors: *Botulinum Toxins--genetics--GE; *Clostridium botulinum --genetics--GE; Amino Acid Sequence; Antibodies, Monoclonal; Base Sequence; Botulinum Toxins--biosynthesis--BI; Clostridium botulinum--pathogenicity --PY; Comparative Study; Genes, Bacterial; Molecular Sequence Data; Peptide Fragments--biosynthesis--BI; Peptide Fragments--genetics--GE; Recombinant Fusion Proteins--biosynthesis--BI; Recombinant Fusion Proteins--genetics --GE; Research Support, Non-U.S. Gov't; Sequence Homology, Nucleic Acid

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Peptide Fragments); 0 (Recombinant Fusion Proteins)

Record Date Created: 19920611
Record Date Completed: 19920611

3/9/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09186518 PMID: 1551445

ADP-ribosylation by Clostridium botulinum **C3 exoenzyme increases steady-state GTPase activities of** recombinant **rhoA and rhoB proteins.**

Mohr C; Koch G; Just I; Aktories K
Rudolf-Buchheim-Institut fur Pharmakologie, Universitat Giessen, Germany.

FEBS letters (NETHERLANDS) Feb 3 1992, 297 (1-2) p95-9, ISSN 0014-5793--Print Journal Code: 0155157
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

ADP-ribosylation of **recombinant** rhoA and rhoB proteins by Clostridium **botulinum** C3 exoenzyme increased steady-state GTP hydrolysis by 50 to 80%. ADP-ribosylation and increase in GTP hydrolysis occurred at similar concentrations of C3, depended on the presence of NAD and were prevented by anti-C3 antibody or heat inactivation of C3. In contrast, GTP hydrolysis by Ile-41 rhoA or Ha-ras, which are no substrates for the transferase, were not affected by C3. ADP-ribosylation facilitated the [3H]GDP release and subsequently, the binding of [3H]GTP to rhoA. The data indicate that the increase in the steady-state GTPase activity by ADP-ribosylation is caused by increasing the rate of GDP release which is suggested to be the rate limiting step of the GTPase cycle of the small GTP-binding proteins.

Descriptors: *ADP Ribose Transferases--metabolism--ME; *Adenosine Diphosphate Ribose--metabolism--ME; *Botulinum Toxins; *Clostridium botulinum--enzymology--EN; *GTP Phosphohydrolases--metabolism--ME; *GTP-Binding Proteins--metabolism--ME; *Membrane Proteins--metabolism--ME;

Guanosine Triphosphate--metabolism--ME; Recombinant Proteins--metabolism --ME; Research Support, Non-U.S. Gov't; rhoA GTP-Binding Protein; rhoB GTP-Binding Protein
CAS Registry No.: 0 (Botulinum Toxins); 0 (Membrane Proteins); 0 (Recombinant Proteins); 20762-30-5 (Adenosine Diphosphate Ribose); 86-01-1 (Guanosine Triphosphate)
Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP Phosphohydrolases); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rhoA GTP-Binding Protein); EC 3.6.5.2 (rhoB GTP-Binding Protein)
Record Date Created: 19920430
Record Date Completed: 19920430

3/9/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08639281 PMID: 2123802
Interaction of recombinant rho A GTP-binding proteins with photoexcited rhodopsin.
Wieland T; Ulibarri I; Gierschik P; Hall A; Aktories K; Jakobs K H
Pharmakologisches Institut, Universitat Heidelberg, FRG.
FEBS letters (NETHERLANDS) Nov 12 1990, 274 (1-2) p111-4, ISSN 0014-5793--Print Journal Code: 0155157
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The small molecular mass GTP-binding proteins rho A, B and C are targets for ADP-ribosyltransferase activity of the **botulinum** exoenzyme C3. The possible interaction of **recombinant** rho A proteins expressed in E. coli with photoexcited rhodopsin was studied by reconstitution with bovine rod outer segment (ROS) membranes depleted of endogenous GTP-binding proteins by treatment with urea. As reported for C3 substrates present in untreated ROS membranes, ADP-ribosylation of recombinant rho A proteins, both normal and Val-14 mutant, by C3 was inhibited when reconstituted with illuminated compared to dark-adapted ROS membranes pretreated with urea. GDP reduced the light-induced inhibition, while GTP[S] and light inhibited ADP-ribosylation of rho A proteins in a synergistic manner.
Descriptors: *GTP-Binding Proteins--metabolism--ME; *Rhodopsin --metabolism--ME; Adenosine Diphosphate Ribose--metabolism--ME; Animals; Cattle; GTP-Binding Proteins--genetics--GE; Guanosine Diphosphate --metabolism--ME; Humans; Light; Protein Binding; Recombinant Proteins --metabolism--ME; Research Support, Non-U.S. Gov't; Rod Outer Segments --metabolism--ME; Transducin--metabolism--ME; rhoA GTP-Binding Protein
CAS Registry No.: 0 (Recombinant Proteins); 146-91-8 (Guanosine Diphosphate); 20762-30-5 (Adenosine Diphosphate Ribose); 9009-81-8 (Rhodopsin)
Enzyme No.: EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.1.- (Transducin); EC 3.6.5.2 (rhoA GTP-Binding Protein)
Record Date Created: 19910124
Record Date Completed: 19910124

3/9/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08053136 PMID:. 2497029

Identification of rho as a substrate for botulinum toxin C3-catalyzed ADP-ribosylation.

Quilliam L A; Lacal J C; Bokoch G M

Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

FEBS letters (NETHERLANDS) Apr 24 1989, 247 (2) p221-6, ISSN 0014-5793--Print Journal Code: 0155157

Contract/Grant No.: GM39434; GM; NIGMS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

Recombinant Aplysia rho and a GTP-binding protein purified from human neutrophil membranes (G22K) were ADP-ribosylated by botulinum toxin C3 with stoichiometries of 0.8 and 0.6, respectively. Rho and G22K appeared to be different proteins since (i) rho migrated faster on polyacrylamide gels, (ii) unlike G22K, rho did not require the presence of cytosol to be ADP-ribosylated, (iii) G22K was not recognized by an anti-rho antiserum, and (iv) antibody 142-24E05 recognized G22K effectively but only poorly cross reacted with rho. ADP-ribosylation had no effect on the ability of rho to bind or hydrolyse GTP. Therefore, it appears that there are multiple botulinum toxin C3 substrates and that the toxin exerts its effects on cell function by a mechanism other than modulating the GTPase activity of rho.

Descriptors: *Botulinum Toxins--metabolism--ME; *GTP-Binding Proteins --metabolism--ME; *Membrane Proteins--metabolism--ME; Adenosine Diphosphate Ribose--metabolism--ME; Animals; Antibodies, Monoclonal; Aplysia; Cattle; Cell Membrane--analysis--AN; Comparative Study; Electrophoresis, Polyacrylamide Gel; Guanosine 5'-O-(3-Thiotriphosphate); Guanosine Triphosphate--analogs and derivatives--AA; Guanosine Triphosphate --metabolism--ME; Humans; Hydrolysis; Kinetics; Molecular Weight; Neutrophils--analysis--AN; Recombinant Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.; Thionucleotides--metabolism--ME; rhoB GTP-Binding Protein

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Membrane Proteins); 0 (Recombinant Proteins); 0 (Thionucleotides); 20762-30-5 (Adenosine Diphosphate Ribose); 37589-80-3 (Guanosine 5'-O-(3-Thiotriphosphate)); 86-01-1 (Guanosine Triphosphate)

Enzyme No.: EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rhoB GTP-Binding Protein)

Record Date Created: 19890616

Record Date Completed: 19890616

3/9/16 (Item 16 from file: 155)

07990931 PMID: 2492192

The rho gene product expressed in E. coli is a substrate of botulinum ADP-ribosyltransferase C3.

Aktories K; Braun U; Rosener S; Just I; Hall A

Rudolf-Buchheim-Institut fur Pharmakologie, Giessen, FRG.

Biochemical and biophysical research communications (UNITED STATES) Jan 16 1989, 158 (1) p209-13, ISSN 0006-291X--Print Journal Code: 0372516

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

The ras-related rho A protein expressed in E. coli, was ADP-ribosylated by botulinum ADP-ribosyltransferase C3. C3 also modified the valine-14 mutant rho protein but not the products of H-ras, R-ras, ral, ypt, and rap 1 genes. A ras-rho chimaera consisting of 60 amino acids from the amino terminus of ras fused to 133 amino acids from the carboxy terminus of rho was not modified by C3. Antibodies raised against the porcine brain cytosolic substrate of C3 cross reacted with the rho, valine-14 rho and ras-rho proteins, but not with the gene products of H-ras, R-ras, ral or rap 1. Polyclonal anti-H-ras antibodies cross reacted with H-ras but not with ral, rho, or the C3 substrate purified from porcine brain.

Descriptors: *ADP Ribose Transferases--metabolism--ME; *Botulinum Toxins; *Escherichia coli--genetics--GE; *GTP-Binding Proteins--metabolism--ME; *Membrane Proteins--metabolism--ME; Clostridium botulinum--enzymology--EN; GTP-Binding Proteins--genetics--GE; Membrane Proteins--genetics--GE; Proto-Oncogene Proteins--metabolism--ME; Proto-Oncogene Proteins p21(ras); Recombinant Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; Substrate Specificity; rhoA GTP-Binding Protein

CAS Registry No.: 0 (Botulinum Toxins); 0 (Membrane Proteins); 0 (Proto-Oncogene Proteins); 0 (Recombinant Proteins)

Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (Proto-Oncogene Proteins p21(ras)); EC 3.6.5.2 (rhoA GTP-Binding Protein)

Record Date Created: 19890217
Record Date Completed: 19890217

? t s3/3,kwic/43

3/9/21 (Item 5 from file: 73)

DIALOG(R)File 73:EMBASE

05440098 EMBASE No: 1993208197

Similarity in nucleotide sequence of the gene encoding nontoxic component of botulinum toxin produced by toxigenic Clostridium butyricum strain BL6340 and Clostridium botulinum type E strain Mashike

Fujii N.; Kimura K.; Yokosawa N.; Oguma K.; Yashiki T.; Takeshi K.; Ohyama T.; Isogai E.; Isogai H.

Department of Microbiology, School of Medicine, Sapporo Medical University, South-1, West-17,Chuo-ku, Sapporo 060 Japan

Microbiology and Immunology (MICROBIOL. IMMUNOL.) (Japan) 1993, 37/5 (395-398)

CODEN: MIIMD ISSN: 0385-5600

DOCUMENT TYPE: Journal; Article

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor toxin is determined in **recombinant** plasmid pU9BUH containing about 6.0 kb HindIII fragment obtained from chromosomal DNA of Clostridium butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The molecular weight calculated from deduced amino acid residues is estimated 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinum type E strain Mashike. This corresponds to the difference of 17 amino acid residues in this nontoxic component.

DRUG DESCRIPTORS:
*botulinum toxin

MEDICAL DESCRIPTORS:
*clostridium botulinum; *clostridium butyricum
article; molecular cloning; nonhuman; nucleotide sequence
SECTION HEADINGS:
  004 Microbiology: Bacteriology, Mycology, Parasitology and Virology

3/9/31 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0008898574 BIOSIS NO.: 199396062990
Similarity in nucleotide sequence of the gene encoding nontoxic component of botulinum toxin produced by toxigenic Clostridium butyricum strain BL6340 and Clostridium botulinum type E strain Mashike
AUTHOR: Fujii Nobuhiro (Reprint); Kimura Kouichi; Yokosawa Noriko; Oguma Keiji; Yashiki Teruo; Takeshi Kouichi; Ohyama Touru; Isogai Emiko; Isogai Hiroshi
AUTHOR ADDRESS: Dep. Microbiol., Sch. Med., Sapporo Med. University, South-1, West-17, Chuo-ku, Sapporo, Hokkaido 060, Japan**Japan
JOURNAL: Microbiology and Immunology 37 (5): p395-398 1993
ISSN: 0385-5600
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor toxin is determined in **recombinant** plasmid pU9BUH containing about 6.0 kb HindIII fragment obtained from chromosomal DNA of Clostridium butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The molecular weight calculated from deduced amino acid residues is estimated 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinum type E strain Mashike. This corresponds to the different of 17 amino acid residues in these nontoxic component.

REGISTRY NUMBERS: 148426-46-4: D12739
DESCRIPTORS:
  MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Genetics; Infection; Physiology
  BIOSYSTEMATIC NAMES: Bacteria--Microorganisms; Endospore-forming Gram-Positives--Eubacteria, Bacteria, Microorganisms; Organisms--Organisms
  ORGANISMS: Gram negative bacteria (Bacteria); endospore-forming gram-positive rods and cocci (Endospore-forming Gram-Positives); Paracoccus denitrificans (Organisms)
  COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Organisms
MOLECULAR SEQUENCE DATABANK NUMBER: D12739--Genbank
  MISCELLANEOUS TERMS: HOMOLOGY; MOLECULAR EVOLUTION
CONCEPT CODES:
  10010 Comparative biochemistry
  10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
  10064 Biochemistry studies - Proteins, peptides and amino acids
  10506 Biophysics - Molecular properties and macromolecules
  31000 Physiology and biochemistry of bacteria
  31500 Genetics of bacteria and viruses
  36002 Medical and clinical microbiology - Bacteriology
BIOSYSTEMATIC CODES:
  05000 Bacteria

07810 Endospore-forming Gram-Positives
00500 Organisms

3/9/36 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0008131076 BIOSIS NO.: 199243099667
MONOCLONAL ANTIBODIES TO BOTULINUM **TOXIN PRODUCED BY** RECOMBINANT **TECHNOLOGY**
AUTHOR: MIDDLEBROOK J L (Reprint); LEATHERMAN D L; SMITH T; CROWELL J
AUTHOR ADDRESS: DEP TOXINOL, PATHOPHYSIOL DIV, US ARMY MED RES INST INFECT DIS, FREDERICK, MD 21702, USA**USA
JOURNAL: Toxicon 30 (5-6): p535 1992
CONFERENCE/MEETING: TENTH WORLD CONGRESS ON ANIMAL, PLANT AND MICROBIAL TOXINS, SINGAPORE, SINGAPORE, NOVEMBER 3-8, 1991. TOXICON.
ISSN: 0041-0101
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
DESCRIPTORS: ABSTRACT CLOSTRIDIUM-BOTULINUM ESCHERICHIA-COLI VACCINE NEUROTOXIN
DESCRIPTORS:
MAJOR CONCEPTS: Immune System--Chemical Coordination and Homeostasis; Infection; Nervous System--Neural Coordination; Pharmacology; Physiology; Toxicology
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Endospore-forming Gram-Positives--Eubacteria, Bacteria, Microorganisms
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10068 Biochemistry studies - Carbohydrates
20506 Nervous system - Pathology
22018 Pharmacology - Immunological processes and allergy
22501 Toxicology - General and methods
22505 Toxicology - Antidotes and prevention
31000 Physiology and biochemistry of bacteria
34502 Immunology - General and methods
34504 Immunology - Bacterial, viral and fungal
36002 Medical and clinical microbiology - Bacteriology
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae
07810 Endospore-forming Gram-Positives

3/9/43 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01384558 ORDER NO: AAD94-31554
THE CRYSTAL STRUCTURES OF HUMAN AND RAT CLARA CELL PHOSPHOLIPID BINDING PROTEINS AND THE PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF BOTULINUM E NEUROTOXIN AND TETANUS TOXIN C-FRAGMENT
Author: UMLAND, TIMOTHY CHARLES
Degree: PH.D.
Year: 1994

Corporate Source/Institution: UNIVERSITY OF PITTSBURGH (0178)
Chairperson: MARTIN SAX
Source: VOLUME 55/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 2551. 239 PAGES
Descriptors: BIOLOGY, MOLECULAR; CHEMISTRY, BIOCHEMISTRY
Descriptor Codes: 0307; 0487

X-ray crystallography was used to determine the three dimensional structures of rat Clara cell phospholipid binding protein and of two crystal forms of human Clara cell phospholipid binding protein-(phosphatidylcholine/phosphatidylinositol) complex. The rat protein was determined to 3.0 A resolution. The P1 crystal form of the human protein was determined to 2.3 A resolution, and the P222 crystal form was determined to 1.9 A resolution.

The Clara cell phospholipid binding protein is secreted specifically by the Clara cells into the extracellular bronchiolar lining layer. The Clara cell predominates in the distal airways of mammalian lung and is responsible for repair and renewal of the epithelium of these airways. The properties of this protein include an ability to inhibit phospholipase A$\sb2$, act as a substrate for transglutaminase, and binding polychlorinated biphenyls. The rat homologue also is capable of binding progesterone. The work reported in this dissertation demonstrated that the human Clara cell phospholipid binding protein binds in vivo phosphatidylcholine and phosphatidylinositol in its large internal hydrophobic cavity. The rat homologue was predicted to have this same property, based on structural similarities. This protein was originally named Clara cell 10 kDa protein (CC10), but it is now more appropriately referred to as Clara cell phospholipid binding protein (CCPBP).

The crystal structures were solved using the molecular replacement method. The structure of rabbit uteroglobin was used as a search model in the determination of the rat CCPBP structure. The determination of the HCCPBP structures employed either rat or human CCPBP search models. Dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylinositol were built into and refined in both human CCPBP structures.

This dissertation also includes the crystallization and preliminary crystallographic analysis of **botulinum** type E neurotoxin (**BoNT** -E) and of **recombinant** tetanus toxin C-fragment (rTTC). **BoNT** -E crystallizes in space group P2, with a = 81.60 A, b = 172.87 A, c = 139.13 A, and β = 98.65$\sp\circ$, with two molecules in the asymmetric unit. rTTC crystallizes in space group P2$\sb1$2$\sb1$2$\sb1$, with a = 79.72 A, b = 93.98 A, and c = 71.30 A. One rTTC molecule resides in the asymmetric unit.

3/9/46 (Item 1 from file: 94)

01870987 JICST ACCESSION NUMBER: 93A0557383 FILE SEGMENT: JICST-E
Similarity in Nucleotide Sequence of the Gene Encoding Nontoxic Component of Botulinum Toxin Produced by Toxigenic Clostridium butyricum Strain BL6340 and Clostridium botulinum Type E Strain Mashike.
FUJII N (1); KIMURA K (1); YOKOSAWA N (1); OGUMA K (1); YASHIKI T (2); TAKESHI K (3); OHYAMA T (3); ISOGAI E (4); ISOGAI H (5)
(1) Sapporo Medical Univ., Hokkaido, JPN; (2) Hokkaido Univ., Hokkaido, JPN ; (3) Hokkaido Inst. Public Health, Hokkaido, JPN; (4)Higashi Nippon Gakuen Univ., Hokkaido, JPN; (5) Sapporo Medical Coll., Hokkaido, JPN
Microbiol Immunol, 1993, VOL.37,NO.5, PAGE.395-398, FIG.1, REF.10
JOURNAL NUMBER: F0715ABF ISSN NO: 0385-5600
UNIVERSAL DECIMAL CLASSIFICATION: 579.222
LANGUAGE: English COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Journal

ARTICLE TYPE: Short Communication
MEDIA TYPE: Printed Publication
ABSTRACT: The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor toxin is determined in **recombinant** plasmid pU9BUH containing about 6.0 kb HindIII fragment obtained from chromosomal DNA of Clostridium butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The molecular weight calculated from deduced amino acid residues is estimated 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinu type E strain Mashike. This corresponds to the difference of 17 amino acid residues in these nontoxic component. (author abst.)
DESCRIPTORS: Clostridium botulinum; botulinus toxin; Clostridium butyricum; nucleotide sequence; amino acid sequence; gene cloning; neurotoxin; precursor(substance)
BROADER DESCRIPTORS: Clostridium; Bacillaceae; endospore-forming rods and cocci; bacterium; microorganism; exotoxin; bacterial toxin; microorganism toxin; poison; toxic substance; matter; primary structure ; structure; genetic information; information; sequence and arrangement ; molecular structure; gene manipulation; genetic technique; technology ; operation(processing)
CLASSIFICATION CODE(S): EG03020N
? logoff hold

3/3,KWIC/43 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01384558 ORDER NO: AAD94-31554
THE CRYSTAL STRUCTURES OF HUMAN AND RAT CLARA CELL PHOSPHOLIPID BINDING PROTEINS AND THE PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF BOTULINUM E NEUROTOXIN AND TETANUS TOXIN C-FRAGMENT
Author: UMLAND, TIMOTHY CHARLES
Degree: PH.D.
Year: 1994
Corporate Source/Institution: UNIVERSITY OF PITTSBURGH (0178)
Source: VOLUME 55/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 2551. 239 PAGES

...both human CCPBP structures.
This dissertation also includes the crystallization and preliminary crystallographic analysis of **botulinum** type E neurotoxin (**BoNT** -E) and of **recombinant** tetanus toxin C-fragment (rTTC). **BoNT** -E crystallizes in space group P2, with a = 81.60 A, b = 172.87 A...
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29sep06 11:34:19 User228206 Session D2642.5
$0.46 0.135 DialUnits File155
$3.52 16 Type(s) in Format 9
$3.52 16 Types
$3.98 Estimated cost File155
$0.11 0.022 DialUnits File349
$0.11 Estimated cost File349
$0.50 0.045 DialUnits File73
$3.10 1 Type(s) in Format 9
$3.10 1 Types
$3.60 Estimated cost File73
$0.40 0.067 DialUnits File5
$4.40 2 Type(s) in Format 9
$4.40 2 Types

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      $0.12  Estimated cost File16
             $0.12    0.022 DialUnits File348
      $0.12  Estimated cost File348
             $0.28    0.067 DialUnits File35
                $2.30  1 Type(s) in Format  9
                $0.10  1 Type(s) in Format 95 (KWIC)
             $2.40  2 Types
      $2.68  Estimated cost File35
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      $1.43  Estimated cost File94
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      $0.26  TELNET
     $19.31  Estimated cost this search
     $19.31  Estimated total session cost   0.650 DialUnits

Logoff: level 05.12.03 D  11:34:19

You are now logged off
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09253478 PMID: 1601841

ADP-ribosylation of the ras-related, GTP-binding protein RhoA inhibits lymphocyte-mediated cytotoxicity.

Lang P; Guizani L; Vitte-Mony I; Stancou R; Dorseuil O; Gacon G; Bertoglio J

Institut National de la Sante et de la Recherche Medicale (INSERM), Unite 333, Institut Gustave Roussy, Villejuif, France.

Journal of biological chemistry (UNITED STATES) Jun 15 1992, 267 (17) p11677-80, ISSN 0021-9258--Print Journal Code: 2985121R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

The Rho proteins are identified as a subgroup of the Ras superfamily of low molecular weight GTP-binding proteins. We have studied the expression of these proteins in human cytotoxic natural killer cells and found that RhoA is the most abundantly expressed member of the Rho family. The Rho proteins are specific substrates for ADP-ribosylation catalyzed by the C3 exoenzyme from Clostridium **botulinum** . We report here that introduction of **recombinant** C3 in electropermeabilized natural killer cells or in cytotoxic T lymphocytes resulted in a dose-dependent inhibition of their cytolytic function. Furthermore, a single substrate is efficiently ADP-ribosylated by C3 in extracts from cytotoxic cells. Biochemical analyses indicate that this substrate is RhoA, and subcellular fractionation experiments demonstrate that it is essentially present in the cytosol of the cells. Western blot analysis, however, revealed that a small proportion of the Rho protein can be found associated with the cell membrane as well as with the cytotoxic granules. These results indicate that the low molecular weight GTP-binding protein RhoA is present in cytotoxic lymphocytes and plays a critical role in cell-mediated cytotoxicity.

Descriptors: *Adenosine Diphosphate Ribose--metabolism--ME; *Botulinum Toxins; *Cytotoxicity, Immunologic; *GTP-Binding Proteins--phys

09220648 PMID: 1577256

Cloning of a Clostridium botulinum type B toxin gene fragment encoding the N-terminus of the heavy chain.

Jung H H; Rhee S D; Yang K H

Department of Life Science, Korea Advanced Institute of Science and Technology, Taejon, Korea.

FEMS microbiology letters (NETHERLANDS) Feb 1 1992, 70 (1) p69-72, ISSN 0378-1097--Print Journal Code: 7705721

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

Two lambda gt11 clones of the toxin gene of Clostridium botulinum type B were identified by the monoclonal antibody specific to the heavy chain of type B toxin. Neither of the expressed fusion proteins from the lysates of lysogenic E. coli Y1089 showed any botulinal toxic activity. One of the clones hybridized to the oligonucleotide probe which was synthesized according to the amino acid sequence of N-terminus of heavy chain. The sequence analysis revealed that highly homologous regions in N-terminus of heavy chain exist among botulinum neurotoxins (type A, B) and tetanus toxin on the amino acid sequence level.

Descriptors: *Botulinum Toxins--genetics--GE; *Clostridium botulinum --genetics--GE; Amino Acid Sequence; Antibodies, Monoclonal; Base Sequence; Botulinum Toxins--biosynthesis--BI; Clostridium botulinum--pathogenicity --PY; Comparative Study; Genes, Bacterial; Molecular Sequence Data; Peptide Fragments--biosynthesis--BI; Peptide Fragments--genetics--GE; Recombinant Fusion Proteins--biosynthesis--BI; Recombinant Fusion Proteins--genetics --GE; Research Support, Non-U.S. Gov't; Sequence Homology, Nucleic Acid

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Peptide Fragments); 0 (Recombinant Fusion Proteins)

Record Date Created: 19920611

Record Date Completed: 19920611

0008898574 BIOSIS NO.: 199396062990

Similarity in nucleotide sequence of the gene encoding nontoxic component of botulinum toxin produced by toxigenic Clostridium butyricum strain BL6340 and Clostridium botulinum type E strain Mashike

AUTHOR: Fujii Nobuhiro (Reprint); Kimura Kouichi; Yokosawa Noriko; Oguma Keiji; Yashiki Teruo; Takeshi Kouichi; Ohyama Touru; Isogai Emiko; Isogai Hiroshi

AUTHOR ADDRESS: Dep. Microbiol., Sch. Med., Sapporo Med. University, South-1, West-17, Chuo-ku, Sapporo, Hokkaido 060, Japan**Japan

JOURNAL: Microbiology and Immunology 37 (5): p395-398 1993

ISSN: 0385-5600

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor toxin is determined in **recombinant** plasmid pU9BUH containing about 6.0 kb HindIII fragment obtained from chromosomal DNA of Clostridium butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The molecular weight calculated from deduced amino acid residues is estimated 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinum type E strain Mashike. This corresponds to the different of 17 amino acid residues in these nontoxic component.

REGISTRY NUMBERS: 148426-46-4: D12739

DESCRIPTORS:

MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Genetics; Infection; Physiology

BIOSYSTEMATIC NAMES: Bacteria--Microorganisms; Endospore-forming Gram-Positives--Eubacteria, Bacteria, Microorganisms; Organisms--Organisms

ORGANISMS: Gram negative bacteria (Bacteria); endospore-forming gram-positive rods and cocci (Endospore-forming Gram-Positives); Paracoccus denitrificans (Organisms)

COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Organisms

MOLECULAR SEQUENCE DATABANK NUMBER: D12739--Genbank

MISCELLANEOUS TERMS: HOMOLOGY; MOLECULAR EVOLUTION

CONCEPT CODES:

09593572 PMID: 8458345

A chimeric toxin to study the role of the 21 kDa GTP binding protein rho in the control of actin microfilament assembly.

Aullo P; Giry M; Olsnes S; Popoff M R; Kocks C; Boquet P

Unite des Toxines Microbiennes URA CNRS 557, Institut Pasteur, Paris, France.

EMBO journal (ENGLAND) Mar 1993, 12 (3) p921-31, ISSN 0261-4189--Print Journal Code: 8208664

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

We have developed a new tool for studying the role of rho in actin stress fibre formation. Clostridium botulinum exoenzyme C3 which affects actin microfilament assembly by ADP-ribosylation of p21 rho was genetically fused in various ways to diphtheria toxin (DT). The resulting chimeric toxins were tested on Vero cells. Chimeras of C3 and both the A and B fragments of diphtheria toxin had reduced cell binding activities but were apparently able to penetrate into Vero cells by the same mechanism as DT. Upon exposure to low pH, DC3B, a fusion protein of C3 and DT B fragment, had a high affinity for the DT receptor, but was apparently not able to translocate to the cytosol upon acidification. In spite of this, addition of picomolar concentrations of DC3B to the growth medium caused disruption of the cell microfilament system associated with vinculin and blocked cell growth efficiently, indicating that the C3 part of DC3B reached the cytosol, albeit by a different mechanism than that of whole diphtheria toxin. The chimeric DC3B toxin was also applied to Vero cells infected by Listeria monocytogenes, a pathogenic bacterium that uses an unknown mechanism of actin polymerization to move rapidly in the cytosol. DC3B inhibited the bacterially induced microfilament assembly indicating that L. monocytogenes utilizes a cellular rho dependent mechanism in this process.

Descriptors: *ADP Ribose Transferases--diagnostic use--DU; *Actins --metabolism--ME; *Botulinum Toxins; *Diphtheria Toxin--diagnostic use--DU; *GTP-Binding Proteins--physiology--PH; *Recombinant Fusion Proteins --diagnostic use--DU; ADP Ribose Transferases--genetics--GE; Animals; Cercopithecus aethiops; Cloning, Molecular; Diphtheria Toxin--genetics--GE; Escherichia coli; Listeria monocytogenes--isolation and purification--IP; Mice; Recombinant Fusion Proteins--genetics--GE; Research Support, Non-U.S. Gov't; Vero Cells; Vinculin--metabolism--ME; rho GTP-Binding Proteins

CAS Registry No.: 0 (Actins); 0 (Botulinum Toxins); 0 (Diphtheria Toxin); 0 (Recombinant Fusion Proteins); 125361-02-6 (Vinculin)

Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.5.2 (rho GTP-Binding Proteins)

Record Date Created: 19930423

Record Date Completed: 19930423

09724873 PMID: 8355622

Similarity in nucleotide sequence of the gene encoding nontoxic component of botulinum toxin produced by toxigenic Clostridium butyricum strain BL6340 and Clostridium botulinum type E strain Mashike.

Fujii N; Kimura K; Yokosawa N; Oguma K; Yashiki T; Takeshi K; Ohyama T; Isogai E; Isogai H

Department of Microbiology, School of Medicine, Sapporo Medical University, Hokkaido, Japan.

Microbiology and immunology (JAPAN) 1993, 37 (5) p395-8, ISSN 0385-5600--Print Journal Code: 7703966

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor toxin is determined in **recombinant** plasmid pU9BUH containing about 6.0 kb HindIII fragment obtained from chromosomal DNA of Clostridium butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The molecular weight calculated from deduced amino acid residues is estimated 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinum type E strain Mashike. This corresponds to the difference of 17 amino acid residues in these nontoxic component.

Descriptors: *Botulinum Toxins--genetics--GE; *Clostridium--genetics--GE; *Clostridium botulinum--genetics--GE; Amino Acid Sequence; Base Sequence; Cloning, Molecular; Clostridium--classification--CL; DNA, Bacterial --genetics--GE; Gene Expression; Molecular Sequence Data; Molecular Weight; Open Reading Frames; Plasmids--genetics--GE; Sequence Homology, Amino Acid; Sequence Homology, Nucleic Acid

Molecular Sequence Databank No.: GENBANK/D12739

CAS Registry No.: 0 (Botulinum Toxins); 0 (DNA, Bacterial); 0 (Plasmids)

Record Date Created: 19930921

Record Date Completed: 19930921

09745618 PMID: 8103915

Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25.

Blasi J; Chapman E R; Link E; Binz T; Yamasaki S; De Camilli P; Sudhof T C; Niemann H; Jahn R

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510.

Nature (ENGLAND) Sep 9 1993, 365 (6442) p160-3, ISSN 0028-0836--Print Journal Code: 0410462

Publishing Model Print; Comment in Nature. 1993 Sep 9;365(6442) 104-5; Comment in PMID 8103914

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

Neurotransmitter release is potently blocked by a group of structurally related toxin proteins produced by Clostridium botulinum. Botulinum neurotoxin type B (BoNT/B) and tetanus toxin (TeTx) are zinc-dependent proteases that specifically cleave synaptobrevin (VAMP), a membrane protein of synaptic vesicles. Here we report that inhibition of transmitter release from synaptosomes caused by botulinum neurotoxin A (BoNT/A) is associated with the selective proteolysis of the synaptic protein SNAP-25. Furthermore, isolated or **recombinant** L chain of **BoNT** /A cleaves SNAP-25 in vitro. Cleavage occurred near the carboxyterminus and was sensitive to divalent cation chelators. In addition, a glutamate residue in the BoNT/A L chain, presumably required to stabilize a water molecule in the zinc-containing catalytic centre, was required for proteolytic activity. These findings demonstrate that BoNT/A acts as a zinc-dependent protease that selectively cleaves SNAP-25. Thus, a second component of the putative fusion complex mediating synaptic vesicle exocytosis is targeted by a clostridial neurotoxin.

Descriptors: *Botulinum Toxins--pharmacology--PD; *Membrane Proteins; *Nerve Tissue Proteins--metabolism--ME; Glutamates--metabolism--ME; Glutamic Acid; In Vitro; Neurotransmitter Agents--metabolism--ME; Research Support, Non-U.S. Gov't; Synaptic Membranes--metabolism--ME; Synaptosomal-Associated Protein 25; Synaptosomes--metabolism--ME; Tetanus Toxin--pharmacology--PD

CAS Registry No.: 0 (Botulinum Toxins); 0 (Glutamates); 0 (Membrane Proteins); 0 (Nerve Tissue Proteins); 0 (Neurotransmitter Agents); 0 (Synaptosomal-Associated Protein 25); 0 (Tetanus Toxin); 56-86-0 (Glutamic Acid)

Record Date Created: 19931008

Record Date Completed: 19931008

09817440 PMID: 8243676

Botulinum neurotoxins serotypes A and E cleave SNAP-25 at distinct COOH-terminal peptide bonds.

Schiavo G; Santucci A; Dasgupta B R; Mehta P P; Jontes J; Benfenati F; Wilson M C; Montecucco C

Centro CNR Biomembrane, Universita di Padova, Italy.

FEBS letters (NETHERLANDS) Nov 29 1993, 335 (1) p99-103, ISSN 0014-5793--Print Journal Code: 0155157

Contract/Grant No.: NS17742; NS; NINDS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

SNAP-25, a membrane-associated protein of the nerve terminal, is specifically cleaved by botulinum neurotoxins serotypes A and E, which cause human and animal botulism by blocking neurotransmitter release at the neuromuscular junction. Here we show that these two metallo-endopeptidase toxins cleave SNAP-25 at two distinct carboxyl-terminal sites. Serotype A catalyses the hydrolysis of the Gln197-Arg198 peptide bond, while serotype E cleaves the Arg180-Ile181 peptide lineage. These results indicate that the carboxyl-terminal region of SNAP-25 plays a crucial role in the multi-protein complex that mediates vesicle docking and fusion at the nerve terminal.

Descriptors: *Botulinum Toxins--metabolism--ME; *Membrane Proteins; *Nerve Tissue Proteins--metabolism--ME; Amino Acid Sequence; Animals; Binding Sites; Brain Chemistry; Hydrolysis; Immunoblotting; Molecular Sequence Data; Nerve Tissue Proteins--chemistry--CH; Peptide Fragments --chemistry--CH; Peptide Fragments--isolation and purification--IP; Peptide Fragments--metabolism--ME; Rats; Recombinant Proteins--metabolism --ME; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.; Synaptosomal-Associated Protein 25; Synaptosomes--chemistry--CH

CAS Registry No.: 0 (Botulinum Toxins); 0 (Membrane Proteins); 0 (Nerve Tissue Proteins); 0 (Peptide Fragments); 0 (Recombinant Proteins); 0 (Snap25 protein, rat); 0 (Synaptosomal-Associated Protein 25)

Record Date Created: 19931229

Record Date Completed: 19931229

10295821 PMID: 7530992

Effect of botulinum C3 exoenzyme on cell growth and cytoskeleton organization in transformed human epidermal cells in culture: a possible role for rho protein in epidermal cells.

Yamamoto M; Morii N; Ikai K; Imamura S

Department of Dermatology, Kyoto University Faculty of Medicine, Japan.

Journal of dermatological science (IRELAND) Oct 1994, 8 (2) p103-9, ISSN 0923-1811--Print Journal Code: 9011485

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

We examined the role of rho gene products (rho proteins) on cell growth and cytoskeleton organization in transformed human epidermal cells in culture (HSC-1), using **recombinant botulinum** C3 exoenzyme which specifically ADP-ribosylates rho proteins. Incubation of HSC-1 cell lysates with C3 exoenzyme revealed a single [32P]ADP-ribosylated protein with a molecular weight of 23,000. This protein was identified as rhoA protein by isoelectric focusing (pI 6.0). Addition of C3 exoenzyme to the culture medium of HSC-1 cells changed the shape of HSC-1 cells to a round form with beaded processes in a time- and dose-dependent manner. Moreover, C3 treatment reduced the cell growth rate; 72-h treatment with C3 exoenzyme at 1, 3, 10, 30 and 60 micrograms/ml culture medium resulted in 9.0 +/- 1.8%, 20 +/- 2.9%, 26 +/- 2.3%, 50 +/- 1.4% and 40 +/- 2.0% inhibition of the growth rate relative to controls, respectively. Under this condition, actin stress fibers were disassembled, as revealed using fluorescent-labeled phallacidin, whereas keratin intermediate filaments were not affected, visualized by immunofluorescence using anti-keratin antibody. These results suggest that rho proteins are closely related to cell growth and that these proteins regulate, at least in part, the assembly of actin stress fibers in transformed human epidermal cells.

Descriptors: *ADP Ribose Transferases--pharmacology--PD; *Botulinum Toxins; *Cytoskeleton--drug effects--DE; *Epidermis--cytology--CY; *Epidermis--physiology--PH; *GTP-Binding Proteins--physiology--PH; Actins --ultrastructure--UL; Adenosine Diphosphate Ribose--metabolism--ME; Cell Division--drug effects--DE; Cell Line, Transformed; Epidermis --ultrastructure--UL; Humans; Keratin--ultrastructure--UL; Research Support, Non-U.S. Gov't; Tumor Cells, Cultured; rhoA GTP-Binding Protein

CAS Registry No.: 0 (Actins); 0 (Botulinum Toxins); 20762-30-5 (Adenosine Diphosphate Ribose); 68238-35-7 (Keratin)

Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 3.6.1.- (GTP-Binding Proteins) ; EC 3.6.5.2 (rhoA GTP-Binding Protein)

Record Date Created: 19950303

Record Date Completed: 19950303

US 20040253673A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0253673 A1**

Williams (43) **Pub. Date: Dec. 16, 2004**

(54) **RECOMBINANT BOTULINUM TOXINS WITH A SOLUBLE C-TERMINAL PORTION**

(75) Inventor: **James A. Williams**, Madison, WI (US)

Correspondence Address:
Frank J. Uxa
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Suite 300
4 Venture
Irvine, CA 92618 (US)

(73) Assignee: **Allergan Sales, Inc., Allergan Botox Limited**, Irvine, CA (US)

(21) Appl. No.: **10/728,696**

(22) Filed: **Dec. 5, 2003**

Related U.S. Application Data

(60) Division of application No. 10/271,012, filed on Oct. 15, 2002.

Continuation of application No. 08/704,159, filed on Aug. 28, 1996.

Continuation-in-part of application No. 08/405,496, filed on Mar. 16, 1995, now Pat. No. 5,919,665.

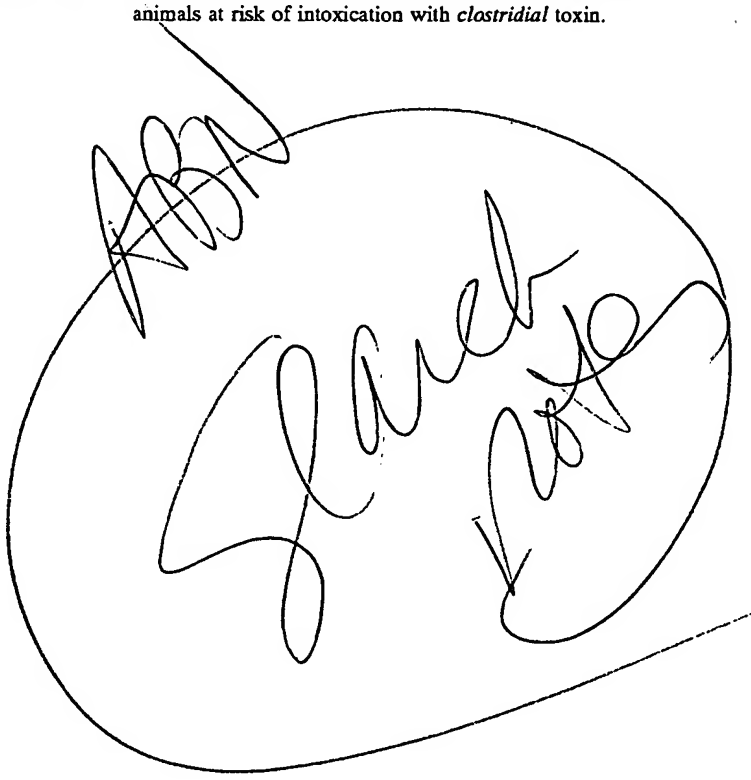
Publication Classification

(51) **Int. Cl.**[7] **C07K 14/33**; C07H 21/04; C12N 1/21

(52) **U.S. Cl.** **435/69.1**; 435/320.1; 435/252.33; 530/350; 536/23.7; 435/348; 435/254.2

(57) **ABSTRACT**

The present invention includes recombinant proteins derived from *Clostridium botulinum* toxins. In particular, soluble recombinant *Clostridium botulinum* type A, type B and type E toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The soluble, endotoxin-free recombinant proteins are used as immunogens for the production of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with *clostridial* toxin.



1-24. (canceled)

25. A recombinant *botulinum* toxin comprising a soluble C-terminal portion of a *botulinum* toxin heavy chain, an N-terminal portion of a *botulinum* toxin heavy chain and a *botulinum* toxin light chain wherein the C-terminal portion comprises SEQ ID NO: 23.

26. The recombinant toxin of claim 25 further comprising a non-toxin protein sequence.

27. The recombinant toxin of claim 25 wherein the toxin is in a solution.

28. The recombinant *botulinum* toxin of claim 25 wherein the light chain is solubilized.

29. The recombinant toxin of claim 25 wherein the C-terminal portion and the N-terminal portion are bonded together.

30. The recombinant toxin of claim 29 wherein the bond is a covalent bond.

31. The recombinant toxin of claim 25 wherein the light chain is bonded to the C-terminal portion or the N-terminal portion.

32. A recombinant *botulinum* toxin comprising a soluble C-terminal portion of a *botulinum* toxin type A heavy chain, an N-terminal portion of a *botulinum* toxin type A heavy chain and a *botulinum* toxin type A light chain wherein the C-terminal portion comprises SEQ ID NO: 23.

33. The recombinant toxin of claim 32 further comprising a non-toxin protein sequence.

34. The recombinant toxin of claim 32 wherein the toxin is in a solution.

35. The recombinant *botulinum* toxin of claim 32 wherein the light chain is solubilized.

36. The recombinant toxin of claim 32 wherein the C-terminal portion and the N-terminal portion are bonded together.

37. The recombinant toxin of claim 36 wherein the bond is a covalent bond.

38. The recombinant toxin of claim 32 wherein the light chain is bonded to the C-terminal portion or the N-terminal portion.

* * * * *

US 20040219637A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2004/0219637 A1

Williams (43) Pub. Date: Nov. 4, 2004

(54) SOLUBLE RECOMBINANT BOTULINUM TOXINS HAVING A C-TERMINAL PORTION OF A HEAVY CHAIN, A N-TERMINAL PORTION OF A HEAVY CHAIN AND A LIGHT CHAIN

(75) Inventor: James A. Williams, Madison, WI (US)

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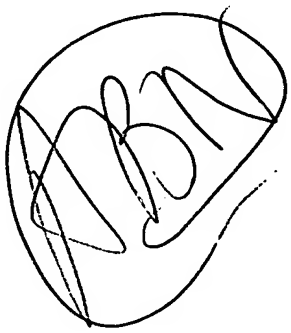
(57) ABSTRACT

The present invention includes recombinant proteins derived from *Clostridium botulinum* toxins. In particular, soluble recombinant *Clostridium botulinum* type A, type B and type E toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The soluble, endotoxin-free recombinant proteins are used as immunogens for the production of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.



1-24. (canceled)

25. A recombinant botulinum toxin comprising a soluble C-terminal portion of a botulinum toxin heavy chain, an N-terminal portion of a botulinum toxin heavy chain and a botulinum toxin light chain 25 wherein the C-terminal portion comprises SEQ ID NO: 23 and the N-terminal portion and the light chain each comprise a portion of SEQ ID NO: 28.

26. The recombinant toxin of claim 25 further comprising a non-toxin protein sequence.

27. The recombinant toxin of claim 25 wherein the toxin is in a solution.

28. The recombinant botulinum toxin of claim 25 wherein the light chain is solubilized.

29. The recombinant toxin of claim 25 wherein the C-terminal portion and the N-terminal portion are bonded together.

30. The recombinant toxin of claim 29 wherein the bond is a covalent bond.

31. The recombinant toxin of claim 25 wherein the light chain is bonded to the C-terminal portion or the N-terminal portion.

32. A recombinant botulinum toxin comprising a soluble C-terminal portion of a botulinum toxin type A heavy chain, an N-terminal portion of a botulinum toxin type A heavy chain and a botulinum toxin type A light chain wherein the C-terminal portion comprises SEQ ID NO: 23 and the N-terminal portion and the light chain each comprise a portion of SEQ ID NO: 28.

33. The recombinant toxin of claim 32 further comprising a non-toxin protein sequence.

34. The recombinant toxin of claim 32 wherein the toxin is in a solution.

35. The recombinant botulinum toxin of claim 32 wherein the light chain is solubilized.

36. The recombinant toxin of claim 32 wherein the C-terminal portion and the N-terminal portion are bonded together.

37. The recombinant toxin of claim 36 wherein the bond is a covalent bond.

38. The recombinant toxin of claim 32 wherein the light chain is bonded to the C-terminal portion or the N-terminal portion.

* * * * *

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pMA maltose Binding Protein

EXPRESSION OF A LARGE, NONTOXIC FRAGMENT OF BOTULINUM NEUROTOXIN SEROTYPE A AND ITS USE AS AN IMMUNOGEN

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H. F. LaPenotiere, M. A. Clayton and J. L. Middlebrook. Expression of a large, nontoxic fragment of botulinum neurotoxin serotype A and its use as an immunogen. *Toxicon* 33, 1383–1386, 1995.—Using the polymerase chain reaction, a large fragment of botulinum toxin was placed in two expression systems, one designed to produce a fusion protein product and another designed to produce only the toxin fragment. Expression of the fragment in the latter system was inconsistent. Expression of the fusion protein was easily measurable by ELISA. Mice were vaccinated with crude fusion protein, then challenged with native toxin. Mice receiving two immunizations were partially protected from up to 1200 LD_{50}, suggesting that this toxin fragment may be a good vaccine candidate to replace the currently used toxoid.

The clostridial neurotoxins are the most toxic substances known to science. The neurotoxin (tetanus toxin) produced by *Clostridium tetani* is encountered by humans as a result of wounds, and remains a serious public health problem in developing countries around the world. Humans are usually exposed to the neurotoxins (botulinum toxins) produced by *Clostridium botulinum* by food poisoning, although there is a rare incidence of wound botulism and a colonizing infection of neonates known as infant botulism (Tacket and Rogawski, 1989). Tetanus poisoning in developed countries is not a widespread public health problem, owing to the availability and widespread use of a safe, effective and inexpensive vaccine. This vaccine is basically a formaldehyde-inactivated culture supernatant from *C. tetani* grown in fermentors. A similar type of vaccine is available to protect from botulinum toxin poisoning (Middlebrook, 1993). However, it suffers from several major problems, most notably, its cost. Since there are seven serotypes of botulinum toxin, complete protection can be afforded only by making seven distinct vaccines and combining them for human administration. Presently, only five of the seven serotypes are represented in the botulinum vaccine. In addition, some of the serotypes are composed of strains that

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do not produce high levels of toxin in culture, and obtaining sufficient toxin for purification and toxoiding is laborious.

We have undertaken the development of a new generation botulinum vaccine through the use of molecular genetics. We used the polymerase chain reaction (PCR) and the native gene of the toxin to prepare a construct encoding a nontoxic 50 kDa carboxy-terminal fragment (H_C) (Niemann, 1992) of the botulinum toxin serotype A. Expressed as a fusion product, this fragment produced immunity in mice when used as an immunogen.

Oligonucleotide primers (Macromolecular Resources, Ft Collins, CO, U.S.A.) incorporating 5′ and 3′ restriction sites of *Bam*HI and *Hind*III, respectively, were used with PCR to amplify the H_C region of *C. botulinum* serotype A toxin (amino acids 861–1296, sequence beginning RLLST). The sequences of the primers were as follows: sense, 5′ TCGAGCTCGGTACCCGGCCGGGGATCCATCGAGGGTAGGAGATTATTATC-TACATTTACTG 3′; antisense, 5′ AGCTCTCAAGCTTACAGTGGCCTTTCTCCCC 3′. PCR reagents were obtained from Perkin-Elmer Cetus (Norwalk, CT, U.S.A.) and the DNA encoding the H_C was from pCBA3, kindly provided by Nigel Minton (Thompson *et al.*, 1990). Expression vectors pMAL-p (New England Biolabs, Beverly, MA, U.S.A.) and pKK233-2 (Pharmacia LKB, Piscataway, NJ, U.S.A.) were used according to the manufacturers' protocols. Gel-purified insert and vector DNA were cleaved with the appropriate restriction enzymes (Life Technologies, Gaithersburg, MD, U.S.A.), purified on low melting point agarose and ligated overnight at 16°C. Competent *Escherichia coli* K12 DH5α host cells (Life Technologies) were transformed according to the supplier's recommendations and plated on LB agar with 100 μg/ml ampicillin. Cells containing either expression vector were grown at 37°C to OD_{600} of 0.4, induced with isopropylthiogalactoside (0.3 mM final concentration) and harvested by centrifugation after 2 hr. The cells were washed, broken by sonication and subjected to centrifugation to produce soluble and particulate fractions. Samples were subjected to SDS gel electrophoresis on precast 11–20% gels (Amersham, Arlington Heights, IL, U.S.A.) at the recommended parameters. No band corresponding to the expected mol. wt for the fragment stood out from control preparations. Attempts to purify the H_c by amylose column procedures were as follows: bacteria were grown to OD_{600} of 0.4, then induced with 3 mM isopropylthiogalactoside for 3 hr at 37°C. Cells were harvested by centrifugation and suspended in lysis buffer with 100 μg/ml Polymixin B. After sonication for 5 min, NaCl was added to 0.5 M, followed by centrifugation. The supernatant was applied to and eluted from the amylose column as per instructions provided by New England Biolabs. SDS gel electrophoresis and Coomassie Blue staining of the partially purified product also did not reveal a specific band at the expected mol. wt. Alternatively, ELISA were performed by first absorbing a capture antibody (horse, monovalent serum raised by immunizing with serotype A toxoid, followed by boost with native serotype A toxin) to the plate overnight at 4°C, then blocking with skim milk for 90 min at 37°C. Various dilutions of the test materials were applied to the plates for 90 min at 37°C, followed by washing and incubation with rabbit anti-botulinum toxin A polyclonal antiserum (furnished by W. H. Lee, U.S.D.A., Beltsville, MD, U.S.A.) for 90 min at 37°C. After washing, goat anti-rabbit antiserum conjugated with horseradish peroxidase (Kirkegaard and Perry, Gaithersburg, MD, U.S.A.) was added and incubated for 90 min at 37°C. Finally, the plate was washed and ABTS (Kirkegaard and Perry) added; plates were read at 405 nm after 20–30 min.

Expression of H_C using pKK233 was variable. In some experiments, ELISA analysis indicated H_C was produced, but in others, none was detected. In contrast, expression in the pMAL system was reliable. Table 1 shows data from an experiment where the pellets

Table 1. Representative ELISA data of fusion protein product (OD_{405})

Dilution factor	Toxin control	pMAL control sup	pMAL control pell	BotHc/pMAL sup # 1	BotHc/pMAL sup # 2	BotHc/pMAL pell # 1	BotHc/pMAL pell # 2	Blank well
102,400	1.77	0.21	0.26	0.47	0.44	0.27	0.29	0.24
25,600	2.07	0.27	0.26	0.87	0.77	0.43	0.43	0.24
6400	2.30	0.23	0.33	1.10	1.19	0.75	0.80	0.22
1600	2.06	0.24	0.35	1.29	1.20	1.17	1.08	0.17
400	2.44	0.32	0.37	1.23	1.37	1.11	1.35	0.14
100	1.99	0.08	0.34	0.69	1.52	1.00	0.24	0.13

and supernatants from lysed *E. coli* cells were evaluated by ELISA. Clearly, there was material expressed by H_C insert-carrying transformed cells that was recognized by antisera to botulinum toxin. The development of color was dilution dependent, and was observed in both the pellet and supernatant fractions. Controls where the vector without H_C insert was used produced color at the intensity level of blank wells.

To evaluate the possibility that the ELISA-reactive material could serve as a protective immunogen, we performed an active immunization experiment with mice. *Escherichia coli* cultures were grown with insert-carrying or insert-free pMAL plasmid. The cells were induced as described, washed and lysed by sonication and the whole-cell preparation was used to vaccinate mice. Mice were vaccinated once or twice, then challenged with toxin; Table 2 shows the results. Mice receiving only one vaccination with an unknown amount of crude H_C survived 3 LD_{50} of toxin, while all of the mice vaccinated with *E. coli* extract died. Mice vaccinated twice with unknown amounts of crude and partially purified H_C likewise were protected (11/11) from a similar challenge dose of toxin, while 6 of 12 control animals succumbed. Challenge of animals with higher doses of toxin produced less clear results. One of three animals challenged with 30 or 300 LD_{50} died, although the symptoms were not those typically exhibited by botulinum toxin-posioned mice, namely labored breathing and hind limb paralysis. Two of three animals challenged with 1200 LD_{50} of toxin survived, while the third animal did exhibit botulinum poisoning symptoms prior to death. Thus, at least partial, perhaps full, protection is seen from up to 300 LD_{50} of botulinum toxin.

Vaccination of animals or humans is currently the best means of protection from poisoning by botulinum toxin(s). However, the vaccine presently available suffers from

Table 2. Response to botulinum neurotoxin type A challenge in mice

Calculated challenge dosage (LD_{50})	One immunization* Control (vector without C fragment insert)	One immunization* Experimental (vector with C fragment insert)	Two immunizations† Control (vector without C fragment insert)	Two immunizations† Experimental (vector with C fragment insert)
3	3/3	0/5	6/12	0/11
30				1/3‡
300				1/3‡
1200				1/3

Results are shown as number of deaths/total.

*Animals received 0.5 ml amylose column-purified product in saline s.c. with Freund's incomplete adjuvant followed by challenge in 2 weeks.

†Animals received equivalent of 100 ml crude sonicated *E. coli* culture lysate resuspended in 0.5 ml saline with Freund's complete adjuvant s.c. at time 0, followed by s.c. boost with amylose-purified product as above at week 2 and challenge at week 4.

‡Deaths were atypical of toxin-associated mortality.

several shortcomings, briefly mentioned above. One possible approach to developing a new vaccine would be to identify a nontoxic fragment of the toxin that would be a good immunogen. Growth, purification and inactivation of the toxin for vaccine purposes is time consuming and expensive, owing to the high hazards associated with handling fully active toxin. If it were possible to start with a nontoxic protein fragment, these hazards and associated costs would be bypassed. In addition, the final product would be much safer, as the potential problem of reversion to toxicity of an inactivated (toxin-derived) product could not occur.

Work with the structurally related tetanus toxin demonstrated that a carboxy-terminal fragment of mol. wt approx. 50,000, known as the C fragment, is a good immunogen and can be prepared using molecular genetics so as not to involve full-sized and fully active toxin (Fairweather *et al.*, 1987; Makoff *et al.*, 1989*a*). It would be of interest to prepare a similar fragment from botulinum toxin and test it as a potential vaccine. Thus far we have been unable to make a corresponding C fragment from botulinum toxin by conventional protein chemistry approaches. Another laboratory has had similar experience (O. Dolly, personal communication). We therefore used PCR to obtain a gene encoding that we defined as the corresponding fragment of botulinum toxin serotype A. Expression of this gene as a fusion protein produced a product which induced immunity in animal experiments. We believe that these data indicate that this fragment is a good candidate for a new generation vaccine against botulinum toxin. Unfortunately, most of the material we expressed was found in the insoluble fraction of our cell preparations. Moreover, the gene appeared to be unstable in at least one other expression system. Therefore, we have elected to attempt construction of a gene encoding the same fragment by synthetic chemistry techniques, in the hope that it might lead to expression of a product that is easier to handle (Makoff *et al.*, 1989*b*).

Acknowledgements—The opinions and assertions contained in this report are personal views of the authors and are not to be construed as official or reflecting the views of the U.S. Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council. We are grateful to J. Edward Brown for performing the ELISA. A preliminary report of some of this work has already appeared (LaPenotiere *et al.*, 1993).

REFERENCES

Fairweather, N. F., Lyness, V. A. and Maskell, D. J. (1987) Immunization of mice against tetanus with fragments of tetanus toxin synthesized in *Escherichia coli*. *Infect. Immun.* **55**, 2541–2545.

LaPenotiere, H., Clayton, M. A., Brown, J. E. and Middlebrook, J. L. (1993) Development of a molecularly engineered vaccine for *C. botulinum* neurotoxins. In: *Proceedings of the International Conference of Clostridial Neurotoxins* (DasGupta, B., Ed.). New York: Plenum Press.

Makoff, A. J., Ballantine, S. P., Smallwood, A. E. and Fairweather, N. F. (1989*a*) Expression of tetanus toxin fragment C in *E. coli*: its purification and potential use as a vaccine. *Bio/Technology* **7**, 1043–1046.

Makoff, A. J., Oxer, M. D., Romanos, M. A., Fairweather, N. F. and Ballantine, S. (1989*b*) Expression of tetanus toxin fragment C in *E. coli*: high level expression by removing rare codons. *Nucl. Acids Res.* **17**, 10,191–10,202.

Middlebrook, J. L. (1993) Contributions of the U.S. Army to botulinum toxin research. In: *Botulinum and Tetanus Neurotoxins: Neurotransmission and Biomedical Aspects*, pp. 515–519 (DasGupta, B., Ed.). New York: Plenum Press.

Niemann, H. (1992) Clostridial neurotoxins—proposal of a common nomenclature. *Toxicon* **30**, 223–225.

Tacket, C. O. and Rogawski, M. A. (1989) Botulism. In: *Botulinum Neurotoxin and Tetanus Toxin*, pp. 351–378 (Simpson, L. L., Ed.). New York: Academic Press.

Thompson, D. F., Brehm, J. K., Oultram, J. D., Swinfield, T. J., Shone, C. C., Atkinson, T., Melling, J. and Minton, N. P. (1990) The complete amino acid sequence of the *Clostridium botulinum* type A neurotoxin, deduced by nucleotide sequence analysis of the encoding gene. *Eur. J. Biochem.* **189**, 78–81.

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(54) **RECOMBINANT BOTULINUM TOXINS WITH A SOLUBLE C-TERMINAL PORTION, AN N-TERMINAL PORTION AND A LIGHT CHAIN**

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(57) **ABSTRACT**

The present invention includes recombinant proteins derived from *Clostridium botulinum* toxins. In particular, soluble recombinant *Clostridium botulinum* type A, type B and type E toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The soluble, endotoxin-free recombinant proteins are used as immunogens for the production of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.

-continued

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          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "DNA"

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

GCCTCGAGTT ATTCTGTCCA TCCTTCATCC AC                                    32


(2) INFORMATION FOR SEQ ID NO: 82:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 12 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: Not Relevant
          (D) TOPOLOGY: Not Relevant

     (ii) MOLECULE TYPE: peptide

     (ix) FEATURE:
          (A) NAME/KEY: Modified-site
          (B) LOCATION: 12
          (D) OTHER INFORMATION: /note= "The asparagine residue at
              this position contains an amide group."

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

Cys Gln Thr Ile Asp Gly Lys Lys Tyr Tyr Phe Asn
1               5                  10
```

1. A host cell containing a recombinant expression vector, said vector encoding a protein comprising at least a portion of a *Clostridium botulinum* toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

2. The host cell of claim 1, wherein and said host cell is capable of expressing said protein at a level greater than or equal to 5% of the total cellular protein.

3. The host cell of claim 1, wherein and said host cell is capable of expressing said protein as a soluble protein at a level greater than or equal to 0.25% of the total soluble cellular protein.

4. The host cell of claim 1, wherein said host cell is an *Escherichia coli* cell.

5. The host cell of claim 1, wherein said host cell is an insect cell.

6. The host cell of claim 1, wherein said host cell is a yeast cell.

7. A host cell containing a recombinant expression vector, said vector encoding a fusion protein comprising a non-toxin protein sequence and at least a portion of a *Clostridium botulinum* toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

8. The host cell of claim 7, wherein said portion of said toxin comprises the receptor binding domain.

9. The host cell of claim 7, wherein said non-toxin protein sequence comprises a poly-histidine tract.

10. A vaccine comprising a fusion protein, said fusion protein comprising a non-toxin protein sequence and at least a portion of a *Clostridium botulinum* toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

11. The vaccine of claim 10 further comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of *Clostridium botulinum* type A toxin.

12. The vaccine of claim 10, wherein said portion of said *Clostridium botulinum* toxin comprises the receptor binding domain.

13. The vaccine of claim 10 wherein said non-toxin protein sequence comprises a poly-histidine tract.

14. The vaccine of claim 10, wherein said vaccine is substantially endotoxin-free.

15. A method of generating antibody directed against a Clostridium botulinum toxin comprising:

a) providing in any order:

i) an antigen comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of a *Clostridium botulinum* toxin, said toxin selected from the group consisting of type B toxin and type E toxin, and

ii) a host; and

b) immunizing said host with said antigen so as to generate an antibody.

16. The method of claim 15, wherein said antigen further comprises a fusion protein comprising a non-toxin protein sequence and at least a portion of *Clostridium botulinum* type A toxin.

17. The method of claim 15, wherein said portion of said *Clostridium botulinum* toxin comprises the receptor binding domain.

18. The method of claim 15 wherein said non-toxin protein sequence comprises a poly-histidine tract.

19. The method of claim 15 wherein said host is a mammal.

20. The method of claim 19 wherein said mammal is a human.

21. The method of claim 15 further comprising step c) collecting said antibodies from said host.

22. The method of claim 21 further comprising step d) purifying said antibodies.

23. The antibody raised according to the method of claim 15.

24. The antibody raised according to the method of claim 16.

* * * * *

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(57) Abstract

This invention is directed to preparation and expression of synthetic genes encoding polypeptides containing protective epitopes of botulinum neurotoxin (BoNT). The invention is also directed to production of immunogenic peptides encoded by the synthetic genes, as well as recovery and purification of the immunogenic peptides from recombinant organisms. The invention is also directed to methods of vaccination against botulism using the expressed peptides.

CLAIMS:

1. A nucleic acid encoding the carboxy-terminal portion of the heavy chain (H_C) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C_1, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, wherein said nucleic acid is expressable in a recombinant organism selected from *Escherichia coli* and *Pichia pastoris*.

2. The nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid sequence selected from SEQ ID No:1 (serotype A), SEQ ID No:7 (serotype B), SEQ ID No:9 (serotype C_1), SEQ ID No:11 (serotype D), SEQ ID No:13 (serotpye E), SEQ ID No:15 (serotype F), and SEQ ID No:17 (serotype G).

3. The nucleic acid of claim 1, wherein the nucleic acid encodes an H_C amino acid sequence of BoNT selected from SEQ ID No:2 (serotype A), SEQ ID No:8 (serotype B), SEQ ID No:10 (serotype C_1), SEQ ID No:12 (serotype D), SEQ ID No:14 (serotpye E), SEQ ID No:16 (serotype F), and SEQ ID No:18 (serotype G).

4. A nucleic acid encoding the amino-terminal portion of the heavy chain (H_N) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype B, BoNT serotype C_1, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, wherein said nucleic acid is expressable in a recombinant organism selected from *Escherichia coli* and *Pichia pastoris*.

5. The nucleic acid of claim 4, wherein said nucleic acid comprises a nucleic acid sequence selected from SEQ ID No:21 (serotype B), SEQ ID No:23 (serotype C_1), SEQ ID No:25 (serotype D), SEQ ID No:27 (serotpye E), SEQ ID No:29 (serotype F), and SEQ ID No:31 (serotype G).

6. The nucleic acid of claim 4. wherein the nucleic acid encodes an H_N amino acid sequence of BoNT selected from SEQ ID No:22 (serotype B), SEQ ID No:24 (serotype C_1), SEQ ID No:26 (serotype D), SEQ ID No:28 (serotpye E), SEQ ID No:30 (serotype F), and SEQ ID No:32 (serotype G).

7. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein the sequence of the nucleic acid is designed by selecting at least a portion of the codons encoding H_C from codons preferred for expression in a host organism.

8. The nucleic acid of claim 7, wherein the host organism is selected from gram negative bacteria, yeast, and mammalian cell lines.

9. The nucleic acid of claim 8, wherein the host organism is *Escherichia coli* or *Pichia pastoris*.

10. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein the nucleic acid sequence encoding H_C is designed by selecting codons encoding H_C which codons provide H_C sequence enriched in guanosine and cytosine residues.

11. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein said nucleic acid is a synthetic nucleic acid.

12. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein said nucleic acid encoding H_C or H_N is expressed in a recombinant host organism with higher yield than a second nucleic acid fragment encoding the same H_C sequence, said second nucleic acid fragment having the wild-type *Clostridum botulinum* sequence of H_C.

13. An expression vector comprising the nucleic acid of any one of claims 1, 3, 4, or 6, whereby H_C or H_N is expressed upon transfection of a host organism with said expression vector.

14. A method of preparing a polypeptide comprising the carboxy-terminal portion of the heavy chain (H_C) of botulinum neurotoxin (BoNT) or the amino-terminal portion of the heavy chain (H_N) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G,

said method comprising culturing a recombinant host organism transfected with the expression vector of claim 13 under conditions wherein H_C or H_N is expressed.

15. The method of claim 14, wherein the recombinant host organism is a eukaryote.

16. The method of claim 14, further comprising recovering insoluble protein from said host organism, whereby a fraction enriched in H_C or H_N is obtained.

17. The method of claim 16, wherein said host organism is *Pichia pastoris*.

18. An immunogenic composition comprising the carboxy-terminal portion of the heavy chain (H_C) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G.

19. The immunogenic composition of claim 18, wherein H_C is prepared by culturing a recombinant organism transfected with an expression vector encoding H_C.

20. The immunogenic composition of claim 19, wherein an insoluble protein fraction enriched in H_C is recovered from said recombinant organism.

21. An immunogenic composition comprising the amino-terminal portion of the heavy chain (H_N) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G.

22. The immunogenic composition of claim 15, wherein H_N is prepared by culturing a recombinant organism transfected with an expression vector encoding H_N.

23. The immunogenic composition of claim 16, wherein an insoluble protein fraction enriched in H_N is recovered from said recombinant organism.

24. An immunogenic composition comprising a polypeptide comprising epitopes contained in the carboxy-terminal portion of the heavy chain (H_C) of botulinum neurotoxin (BoNT) or the amino-terminal portion of the heavy chain (H_N) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, said epitopes eliciting protective immunity toward the respective BoNT serotype.

25. The immunogenic composition of claim 25, wherein said immunogenic composition elicits an ELISA response to the respective BoNT serotype in an animal, said ELISA response being detectable upon 100-fold dilution of serum from said animal.